ELSEVIER

Agricultural and Forest Meteorology 70 (1994) 279–287

AGRICULTURAL AND FOREST METEOROLOGY

Influence of enhanced CO_2 concentration and irrigation on sudangrass digestibility

D.E. Akin*[,a], B.A. Kimball[b], J.R. Mauney[c], R.L. LaMorte[b], G.R. Hendrey[d], K. Lewin[d], J. Nagy[d], R.N. Gates[e]

[a]*Richard B. Russell Agricultural Research Center, ARS–USDA, P.O. Box 5677, Athens, GA 30613, USA*
[b]*US Water Conservation Laboratory, ARS–USDA, 4331 East Broadway Road, Phoenix, AZ 85040, USA*
[c]*Western Cotton Research Laboratory, ARS–USDA, Phoenix, AZ 85040, USA*
[d]*Brookhaven National Laboratory, US DOE, Upton, Long Island, NY 11973, USA*
[e]*Coastal Plain Experiment Station, ARS–USDA, Tifton, GA 31794, USA*

Received 24 June 1992; revision accepted 22 November 1993

Abstract

An experimental line of sudangrass (*Sorghum bicolor* L. Moench) was included in the free-air CO_2 enrichment (FACE) project in 1991 at the University of Arizona Maricopa Agricultural Center to evaluate the effect of ambient (approximately 370 μmol mol^{-1}) and enriched (550 μmol mol^{-1}) CO_2 in well-watered or water-stressed plots. Our specific objective was to determine modifications caused by these environmental effects on the percentages of morphological parts and the fiber components, and on the in vitro digestibility in vegetative and mature harvests. Enrichment with CO_2 did not ($P > 0.05$) change the percentages of morphological parts or fiber components, or the digestibility of any of the morphological components. Protein levels tended to be lower in CO_2-enriched plants. However, water-stressed plants tended to have a higher proportion of leaves (blades and sheaths) and a lower proportion of stems, were more digestible, and had lower amounts of anti-quality, aromatic compounds within the plant cell. Stems had the highest digestibility of all morphological components (about 75% in vegetative plants) despite the lowest levels of protein. Stems also showed the greatest changes caused by all treatments, including a 20% decline in digestibility from vegetative to mature samples. The results indicate that enriching CO_2 to 550 μmol mol^{-1} did not reduce digestibility of sudangrass.

* Corresponding author.

Elsevier Science B.V.
SSDI 0168-1923(93)05040-Y

1. Introduction

Studies indicate that atmospheric concentrations of CO_2 are steadily increasing (Bacastow et al., 1985). Some reports suggest that current concentrations of about 350 μmol mol^{-1} might double during the next century (Houghton et al., 1990). Increased concentrations of CO_2 could have a dramatic effect on plants and, consequently, studies have been conducted in recent years to assess this effect on plant growth and physiology, agricultural yield, and economic consequences (reviewed by Kimball (1983) and Allen (1990)). One probable advantage of increased atmospheric concentrations of CO_2 is that plant yield will be significantly increased. For example, Kimball (1983), in reviewing several studies of many agricultural crops, reported that overall yield may increase by 33% with a doubling of atmospheric CO_2 concentrations.

Despite the advantage of increased yield, little is known about the quality of agricultural commodities that will be produced in an environment of increased CO_2 levels. No results are available on changes in quality of forage for ruminants. The importance of forages to the economy is indicated by statistics, compiled for the USA in 1989, that reported hay production of 131 Mg (tonnes) with an economic value of over $11 billion (US Department of Agriculture, 1990). Further, changes in quality are important, as indicated by evaluations of bermudagrass (*Cynodon dactylon* L. Pers.) cultivars, which showed that a 12% increase in its digestibility resulted in a 30% increase in mean animal weight gain (Lowrey et al., 1968).

Sudangrass is a C_4, drought-tolerant, high-yielding warm season annual grass that provides high-quality pasture, hay, and silage (Ball et al., 1991). Our specific objective was to evaluate changes in quality as a result of CO_2 enrichment for sudangrass grown under wet and dry conditions. We assessed changes in the proportions of morphological parts and their in vitro digestibilities and chemical characteristics related to quality.

2. Materials and methods

2.1. Overall design and procedures

This study was conducted as part of the free-air CO_2 enrichment (FACE) joint project of the US Department of Energy and the US Department of Agriculture, carried out at the University of Arizona Maricopa Agricultural Center in 1991 (Lewin et al., 1994). This design permitted plant growth under specific modifications of parameters in an otherwise natural environment, rather than growth in artificial laboratory conditions. Enriched CO_2 was maintained at 550 μmol mol^{-1} within four circular plots (22 m diameter) from 05:00 to 19:00 h by computer-controlled instrumentation. Four replicate control plots had ambient concentrations of CO_2 at about 370 μmol mol^{-1}. Each of the main CO_2 plots was split with regard to irrigation. Half of each plot was well-watered ('wet'), receiving a total of 701 mm water on a 3–5 day schedule during the 16 week growth period of sudangrass. This amount of irrigation was sufficient to replace the full consumptive use of crops. The other half of each plot was water-stressed ('dry'), receiving a total of 456 mm water over the same 16 week period. The water was applied using a drip irrigation system with the tubes buried about 0.1 m under the rows. Equal applications of urea (about 15 kg ha^{-1} N) were added through the irrigation system to all plots at approximately weekly intervals. The major crop for the study was cotton (*Gossypium hirsutum* L.), and the sudangrass was included in designated areas of each plot. Thus, there were 16 semi-circular plots with two levels of CO_2, two levels of irrigation, and four replicates of each treatment combination.

2.2. Forage samples

An experimental line of dwarf forage sudangrass (1985, RDC) was developed and supplied by W.W. Hanna (Coastal Plain Experiment Station, ARS–USDA, Tifton, GA). Sudangrass seeds were planted about 10 cm apart in two 1 m sections of row in each plot on 29 May, 1991, after young cotton plants were pulled from the rows. Plants were destructively harvested (i.e. all plant material was collected, including roots) twice. In the early harvest, on 22 July, at approximately 8 weeks of age, every third plant within the rows was collected. Most of these plants were vegetative, with only a few having immature seed heads. In the late harvest, on 16 September, at approximately 16 weeks of age, the remaining plants within the rows were collected. These plants were mature, having fully developed seed heads. After harvest, intact plants were placed in glasshouses and air-dried for several weeks. Morphological parts of the dried plants were separated, freeze-dried, and weighed. Roots were washed to remove the soil before freeze-drying. Leaf blades, leaf sheaths, and stems were ground in a Wiley mill to pass a 1 mm screen for fiber and protein determinations and for in vitro evaluation of digestibility.

2.3. Analyses

Single samples from each replicate were analyzed for neutral detergent fiber (NDF) (Van Soest and Wine, 1967), acid detergent fiber (ADF), acid detergent lignin (ADL) (Van Soest, 1963), permanganate lignin (PML) (Van Soest and Wine, 1968), and protein (Association of Official Analytical Chemists (AOAC), 1980). Feed analyses, based on a series of detergent extractions, are used to assess the various chemical fractions of the forage and, thereby, to estimate forage quality and especially digestibility by rumen microorganisms (Van Soest, 1967; Barton et al., 1976). The NDF treatment separates the soluble components from the fiber, thereby providing an estimate of the cell wall content (Van Soest and Wine, 1967). The NDF fraction contains potentially digestible, structural carbohydrates (e.g. cellulose and much of the hemicellulose) and the nondigestible aromatic components (e.g. lignins). Phenolic compounds limit digestibility of the potentially degradable polysaccharides through association with condensed, polymeric aromatics (i.e. lignins) and also through covalently linked phenolic acids. The ADF and ADL treatments remove the less tightly associated structural carbohydrates of the cell wall, thus providing an assessment of the most refractory carbohydrates bound with the aromatic constituents in the cell wall (Akin et al., 1975). The treatments to estimate lignin (i.e. ADL and PML) do not provide a precise characterization of the chemical nature of the aromatic compounds, which probably differ for the two procedures (Van Soest and Wine, 1967).

For in vitro digestibility, the Tilley and Terry (1963) two-stage procedure was used to analyze duplicate samples per replicate. 'Summative digestibilities' were calculated to estimate the digestibility of the shoots. For these results, the percentage of each morphological component was calculated from the total weight of leaf blades, leaf sheaths, and stems. This percentage was multiplied by the digestibility coefficient for each component, and the products for blades, sheaths and stems were summed.

2.4. Statistics

Data were analyzed using the procedure of the Statistical Analysis System Intitute Inc. (1985) for a split plot experiment with repeated measurements. Error term (a), to test the main effect (enriched CO_2 treatment vs ambient), was the residual sum of squares from treatment × ring, with three degrees of freedom. Error term (b) in the subunit analysis was the residual sum of squares.

3. Results

The percentages of total dry weight partitioned among the various morphological

Table 1
Percentage of total dry weight in various morphological components of sudangrass grown in control (ambient — about 370 μmol mol^{-1}) and FACE (enriched to 550 μmol mol^{-1}) CO_2 concentrations and with well-watered (wet) and water-stressed (dry) levels of irrigation

CO_2 Treatment	Irrigation	Percentage morphological components[d]				
		Leaf blades	Leaf sheath	Stems	Flowers	Roots
Early harvest						
Control	Wet	40.8 ± 3.8^{ab}	16.1 ± 1.3^{a}	31.1 ± 3.2^{a}	0.40 ± 0.37^{a}	11.6 ± 2.4^{a}
Control	Dry	46.8 ± 2.3^{c}	18.0 ± 0.4^{b}	22.5 ± 3.2^{b}	0.03 ± 0.05^{b}	12.7 ± 1.3^{a}
FACE	Wet	38.8 ± 2.3^{b}	16.1 ± 1.2^{a}	31.6 ± 3.3^{a}	0.08 ± 0.15^{b}	13.4 ± 1.3^{a}
FACE	Dry	44.9 ± 2.4^{ac}	17.3 ± 0.9^{ab}	25.7 ± 2.5^{b}	0^{b}	12.1 ± 0.9^{a}
Late harvest						
Control	Wet	21.5 ± 3.3^{ab}	11.8 ± 0.5^{a}	55.0 ± 7.6^{a}	6.8 ± 2.2^{a}	5.0 ± 4.7^{a}
Control	Dry	22.3 ± 1.7^{a}	10.8 ± 1.9^{a}	50.5 ± 5.7^{a}	9.8 ± 5.2^{a}	7.0 ± 4.2^{a}
FACE	Wet	17.8 ± 1.0^{b}	10.3 ± 1.0^{a}	55.0 ± 6.3^{a}	12.8 ± 3.8^{a}	4.5 ± 6.4^{a}
FACE	Dry	21.5 ± 3.0^{ab}	11.0 ± 2.4^{a}	51.5 ± 8.2^{a}	10.0 ± 4.3^{a}	5.8 ± 5.9^{a}

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leqslant 0.05$.

parts (Table 1) were not different ($P > 0.05$) as a result of CO_2 treatment, but differences occurred between irrigation treatments. Leaf blades and sheaths were greater ($P \leqslant 0.05$), or tended to be greater, in plants grown under water stress in the early harvest; stems contributed a higher proportion of biomass for well-watered plants in both harvests. In the early harvest, plants were generally pre-flowering, but water stress tended to delay maturity, as shown by a lower proportion of plants having flowers.

In vitro digestibilities for the various morphological parts (Table 2) were not different ($P > 0.05$) with CO_2 treatments, but consistent trends occurred with the irrigation treatments. Leaves and stems grown under water stress were more digested ($P \leqslant 0.05$), or tended to be more digested, than those of plants grown with ample water, and this phenomenon was significant ($P \leqslant 0.05$) in late-harvested stems for both CO_2 levels. The order of digestibility was stems > leaf blades > leaf sheaths for both harvests. When digestibilities of morphological parts were considered for all four treatments, the decline in digestibility from early to late harvest was 20%, 9%, and 18% for stems, blades, and sheaths, respectively.

Fiber and protein concentrations for the various morphological parts of sudangrass were generally not different ($P > 0.05$) with CO_2 treatment (Table 3). Two exceptions were that CO_2 enrichment increased ($P \leqslant 0.05$) NDF in early-harvested leaf blades and decreased ($P \leqslant 0.05$) stem protein in both harvests. Trends, however, were apparent between irrigation treatments, but these varied for the chemical components and for the harvests. For all morphological parts, early-harvested and water-stressed plants had lower ($P \leqslant 0.05$) amounts of ADF, and stems also had lower ($P \leqslant 0.05$) NDF amounts. In the late-harvested plants, the most consistent trend

Table 2
In vitro digestibility of morphological parts of sudangrass grown in control (ambient — about 370 μmol mol^{-1}) or FACE (enriched to 550 μmol mol^{-1}) CO_2 concentrations and with well-watered (wet) or water-stressed (dry) levels of irrigation

CO_2 Treatment	Irrigation	Percentage in vitro digestibility[d]		
		Leaf blade	Leaf sheath	Stem
Early harvest				
Control	Wet	64.9 ± 2.0^{ab}	60.6 ± 1.1^{ab}	74.2 ± 3.3^{a}
Control	Dry	65.8 ± 2.9^{a}	62.1 ± 1.6^{ac}	77.4 ± 3.4^{a}
FACE	Wet	64.7 ± 1.5^{ab}	59.7 ± 0.8^{b}	76.3 ± 2.2^{a}
FACE	Dry	62.5 ± 0.9^{b}	63.9 ± 1.7^{c}	77.6 ± 1.7^{a}
Late harvest				
Control	Wet	56.6 ± 2.3^{a}	51.3 ± 2.4^{a}	59.0 ± 2.6^{a}
Control	Dry	59.4 ± 3.0^{a}	50.9 ± 1.7^{a}	63.8 ± 2.2^{b}
FACE	Wet	58.6 ± 1.7^{a}	50.2 ± 3.3^{a}	59.1 ± 1.2^{a}
FACE	Dry	59.8 ± 1.9^{a}	50.9 ± 2.9^{a}	63.0 ± 3.3^{b}

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leqslant 0.05$.

Table 3
Chemical composition of morphological components of sudangrass grown in control (ambient — about 370 μmol mol^{-1}) or FACE (enriched to 550 μmol mol^{-1}) CO_2 concentrations and with well-watered (wet) or water-stressed (dry) levels of irrigation[d]

CO_2 Treatment	Irrigation	Percentage components in early harvest				
		Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin	Permanganate lignin	Protein
Leaf blade						
Control	Wet	60.7 ± 1.2^{a}	26.4 ± 1.3^{ab}	3.1 ± 0.1^{a}	5.9 ± 1.6^{a}	19.9 ± 1.0^{a}
Control	Dry	60.3 ± 1.3^{a}	24.4 ± 0.5^{c}	3.1 ± 0.2^{a}	4.9 ± 0.2^{a}	18.6 ± 0.8^{a}
FACE	Wet	62.9 ± 1.1^{b}	27.3 ± 0.8^{a}	3.3 ± 0.3^{a}	5.8 ± 0.5^{a}	18.9 ± 1.4^{a}
FACE	Dry	61.6 ± 0.6^{ab}	25.7 ± 1.3^{bc}	3.2 ± 0.2^{a}	5.8 ± 1.7^{a}	19.1 ± 0.7^{a}
Leaf Sheath						
Control	Wet	65.4 ± 0.7^{a}	35.7 ± 0.8^{a}	4.1 ± 0.2^{ab}	7.5 ± 1.0^{a}	6.3 ± 0.2^{a}
Control	Dry	62.7 ± 0.3^{b}	31.6 ± 0.7^{b}	3.9 ± 0.2^{b}	6.2 ± 0.3^{a}	6.2 ± 1.7^{a}
FACE	Wet	64.7 ± 1.9^{ac}	35.4 ± 0.3^{a}	4.9 ± 1.3^{a}	7.0 ± 0.1^{a}	5.6 ± 0.3^{a}
FACE	Dry	63.4 ± 0.6^{bc}	32.5 ± 1.5^{b}	3.9 ± 0.1^{b}	7.1 ± 1.4^{a}	6.2 ± 0.5^{a}
Stem						
Control	Wet	54.5 ± 1.5^{a}	31.0 ± 0.6^{a}	6.0 ± 5.0^{a}	6.1 ± 0.5^{a}	5.1 ± 0.3^{ab}
Control	Dry	48.6 ± 4.2^{b}	25.5 ± 2.5^{b}	4.9 ± 3.8^{a}	4.7 ± 0.4^{b}	8.3 ± 0.8^{c}
FACE	Wet	54.2 ± 2.0^{a}	31.3 ± 1.5^{a}	3.4 ± 0.5^{a}	5.9 ± 0.3^{a}	4.5 ± 0.6^{b}
FACE	Dry	50.6 ± 1.9^{ab}	27.5 ± 1.9^{b}	3.1 ± 0.4^{a}	5.7 ± 0.5^{a}	6.0 ± 0.8^{a}

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leqslant 0.05$.

was in the concentrations of ADF, ADL, and PML, which were lower ($P \leqslant 0.05$), or tended to be lower, for plants grown under water stress. Generally, the NDF and lignin concentrations increased from early-harvested to late-harvested plants for all morphological parts. Although generally not significant ($P > 0.05$), protein concentrations (Table 3) showed a trend for lower values with CO_2 enrichment, and values were lower ($P \leqslant 0.05$) for protein in FACE stems. Protein amounts were lowest in stems, despite the highest digestibility for this part, and amounts were significantly higher ($P \leqslant 0.05$) in water-stressed plants, regardless of CO_2 treatment.

'Summative digestibilities' (Table 4) indicated that CO_2 enrichment did not alter the overall digestibility of plant shoots. With ample water, the percentage decrease in digestibility for early-harvested and late-harvested shoots was 15%, whereas the decrease was only 10% with water-stressed plants, regardless of CO_2 concentration.

4. Discussion

Forages, such as the sudangrass used in the present study, are composed of plant cell walls (i.e. fiber) and soluble components inside the cells. Soluble components (e.g.

Table 4
Summative digestibilities of sudangrass plants grown in control (ambient — about 370 μmol mol^{-1}) and FACE (enriched to 550 μmol mol^{-1}) CO_2 concentrations and with well-watered (wet) or water-stressed (dry) levels of irrigation

CO_2 Treatment	Irrigation	Percentage in vitro digestibility[d]	
		Early harvest	Late harvest
Control	Wet	67.5 ± 2.0^a	57.6 ± 1.6^a
Control	Dry	68.1 ± 1.1^a	61.2 ± 1.9^b
FACE	Wet	67.9 ± 1.1^a	57.9 ± 0.8^a
FACE	Dry	67.2 ± 0.8^a	60.5 ± 2.9^{ab}

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leqslant 0.05$.

sugars and proteins) are virtually 100% digestible, but plant cell walls vary in bioavailability (Van Soest, 1967). Although the amount of cell walls per se can influence digestibility, the greatest limitation to plant digestibility is the association of structural carbohydrates of the cell wall (e.g. hemicellulose) with phenolic compounds (Akin and Chesson, 1989; Hartley and Ford, 1989). Therefore, the amount and type of fiber has a dramatic influence on forage digestibility (Akin, 1989).

The CO_2 treatment did not alter the in vitro digestibility or the chemical components (i.e. aromatics) most limiting cell wall digestibility for sudangrass. Irrigation treatment often altered digestibility, with water-stressed plants tending to have a higher digestibility and lower amounts of phenolic compounds in the fiber. This effect was more pronounced in the stem component. In addition to the fiber components, protein amounts in stems were higher in water-stressed plants, but this component did not appear to have a substantial influence in the in vitro procedure, where N was not limiting. Our data are in agreement with those of Wilson (1982), who, based on studies in the literature assessing environment and digestibility, concluded that light to moderate drought stress increased digestibility compared with that of well-watered plants. The lower contents of stem, lignin, and flowers support the contention of Wilson (1982) that plant aging may be slower in plants receiving less water.

The treatments influenced the digestibility of the stem component more than that of the other components of sudangrass. Although stems are often lower in digestibility than leaves (Pritchard et al., 1963; Twidwell et al., 1991), this component of sudangrass in the present study had the highest digestibility. Other work (D.E. Akin, unpublished data, 1992) indicated that both pith and rind of sudangrass are potentially highly digestible in young stages. Plant cell walls have been reported in general to decline in digestibility with increased maturity (Van Soest, 1967). The steep decline in stem digestibility with maturity found in this study is in agreement with other data showing that maturity reduces stem digestibility to a greater extent than that of leaves (Pritchard et al., 1963).

We are not aware of any previously published work on the effect of CO_2 enrichment on forage digestibility. However, unpublished results (D.E. Akin and H.B. Johnson, 1992) indicated that leaves and stems of wheat (*Triticum aestivum* L.) did not differ in digestibility when produced under ambient (350 μmol mol^{-1}) or subambient (220 and 250 μmol mol^{-1}) concentrations of CO_2; water stress resulted in improved digestibility of stems, regardless of CO_2 concentration. Similarly, preliminary work (D.E. Akin, unpublished data, 1993), with wheat grown in a similar FACE project to that described above, indicated that enriched CO_2 concentrations have little effect on digestibility. A previous study (Kimball et al., 1987), involving beet armyworm herbivory of cotton, led to the conclusion that cotton grown under CO_2 enrichment (650 μmol mol^{-1}) was of lower nutritive value to insects than plants grown under ambient CO_2 concentrations. Further, some plants respond to enhanced CO_2 with increased starch grains and alterations in plant anatomy (Thomas and Harvey, 1983; Vu et al., 1989), both characteristics which potentially influence biodegradability (Akin and Burdick, 1977; Akin, 1989). The present study of sudangrass, using the mixed rumen microbial population that includes microorganisms potentially able to catabolize all carbohydrates within the plant, indicated that the digestibility of this forage was not altered by CO_2 enrichment.

Acknowledgments

We thank Dr W.W. Hanna (Coastal Plain Experiment Station, ARS–USDA, Tifton, GA) for sudangrass seeds, R. Seay (U.S. Water Conservation Laboratory, ARS–USDA, Phoenix, AZ), Dr W.R. Windham (Russell Research Center, ARS–USDA, Athens) GA, for statistical analyses of the results, and D. Smith and G. Coker (Russell Research Center, ARS–USDA, Athens, GA) for technical assistance.

References

Akin, D.E., 1989. Histological and physical factors affecting digestibility of forages. Agron. J., 81: 17–25.

Akin, D.E. and Burdick, D., 1977. Rumen microbial degradation of starch-containing bundle sheath cells in warm-season grasses. Crop Sci., 17: 529–533.

Akin, D.E. and Chesson, A., 1989. Lignification as the major factor limiting forage feeding value especially in warm conditions. Proc. 16th Int. Grassl. Longr., 4–11 October 1989, Nice France. French Grassl. Soc., Paris, pp. 1753–1760.

Akin, D.E., Barton, II, F.E. and Burdick, D., 1975. Scanning electron microscopy of coastal bermuda and Kentucky-31 tall fescue extracted with neutral and acid detergents. J. Agric. Food Chem., 23: 924–927.

Allen, Jr, L.H., 1990. Plant responses to rising carbon dioxide and potential interactions with air pollutants. J. Environ. Qual., 19: 15–34.

Association of Official Analytical Chemists (AOAC), 1980. Official Methods of Analysis, 13th edn. AOAC, Washington, DC, p. 126.

Bacastow, R.B., Keeling, C.D. and Whorf, T.P., 1985. Seasonal amplitude increase in atmospheric CO_2 concentration at Mauna Loa, Hawaii, 1959–1982. J. Geophys. Res., 90: 10529–10540.

Ball, D.M., Hoveland, C.S. and Lacefield, G.D., 1991. Southern Forages. Potash & Phosphate Institute and the Foundation for Agronomic Research, Atlanta, GA, pp. 34, 137.

Barton, II, F.E., Amos, H.E., Burdick, D. and Wilson, R.L., 1976. Relationship of chemical analysis to in vitro digestibility for selected tropical and temperate grasses. J. Anim. Sci., 43: 504–512.

Hartley, R.D. and Ford, C.W., 1989. Phenolic constituents of plant cell walls and wall biodegradability. In: N.G. Lewis and M.G. Paice (Editors), Plant Cell Wall Polymers: Biogenesis and Biodegradation. ACS Symp. Ser. 399. American Chemical Society, Washington, DC, pp. 137–145.

Houghton, J.T., Jenkins, G.J. and Ephramus, J.J. (Editors), 1990. Climate Change. The IPCC Scientific Assessment. Cambridge University Press, New York, 364 pp.

Kimball, B.A., 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. Agron. J., 75: 779–788.

Kimball, B.A., Mauney, J.R., Akey, D.H., Hendrix, D.L., Allen, S.G., Idso, S.B., Radin, J.W. and Lakatos, E.A., 1987. Response of vegetation to carbon dioxide: effects of increasing CO_2 on the growth, water relations and physiology of plants grown under optimal and limiting levels of water and nitrogen. Rep. 049, US Department of Energy, Carbon Dioxide Research Division, and US Department of Agriculture, Agricultural Research Service, Washington, DC, 124 pp.

Lewin, K.F., Hendrey, G.R., Nagy, J. and LaMorte, R.L., 1994. Design and application of a free-air carbon dioxide enrichment facility. Agric. For. Meteorol., 70: 15–29.

Lowrey, R.S., Burton, G.W., Johnson, Jr, J.C., Marchant, W.H. and McCormick, W.C., 1968. In vivo studies with Coastcross 1 and other bermudas. GA Agric. Exp. Sta. Res. Bull., 55: 5–22.

Pritchard, G.I., Folkins, L.P. and Pigden, W.J., 1963. The in vitro digestibility of whole grasses and their parts at progressive stages of maturity. Can. J. Plant Sci., 43: 79–87.

Statistical Analysis Systems Institute Inc., 1985. SAS/STAT Guide for Personal Computers, Version 6. SAS Institute Inc., Cary, NC, pp. 183–260.

Thomas, J.F. and Harvey, C.N., 1983. Leaf anatomy of four species grown under continuous CO_2 enrichment. Bot. Gaz., 144: 303–309.

Tilley, J.M.A. and Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc., 18: 104–111.

Twidwell, E.K., Johnson, K.D., Patterson, J.A., Cherney, J.H. and Bracker, C.E., 1991. Degradation of switchgrass anatomical tissue by rumen microorganisms. Crop Sci., 30: 1321–1328.

US Department of Agriculture, 1990. Agricultural Statistics. US Government Printing Office, Washington, DC, pp. 230–233.

Van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Anal. Chem., 46: 829–835.

Van Soest, P.J., 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Anim. Sci., 26: 119–128.

Van Soest, P.J. and Wine, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J. Assoc. Off. Anal. Chem., 50: 50–55.

Van Soest, P.J. and Wine, R.H., 1968. Determination of lignin and cellulose in acid–detergent fiber with permanganate. J. Assoc. Off. Anal. Chem., 51: 780–785.

Vu, J.C.V., Allen, Jr, L.H. and Bowes, G., 1989. Leaf ultrastructure, carbohydrates and protein of soybeans grown under CO_2 enrichment. Environ. Exp. Bot., 29: 141–147.

Wilson, J.R., 1982. Environmental and nutritional factors affecting herbage quality. In: J.B. Hacker (Editor), Nutritional Limits to Animal Production from Pastures. Commonwealth Agricultural Bureaux, Slough, UK, pp. 111–131.